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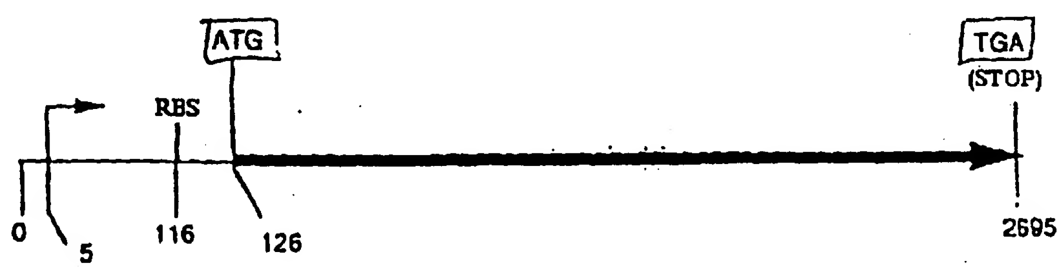

(57) Abstract

The invention features Helicobacter ClpB polypeptides, and methods and compositions for preventing and/or treating Helicobacter infection using these polypeptides. The figure shows a schematic representation of the nucleotide sequence of the insert of plasmid pCP6 which encodes Helicobacter ClpB.

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HELICOBACTER CLPB

Background of the Invention

This invention relates to methods and compositions for preventing and/or treating *Helicobacter* infection.

Helicobacter is a genus of spiral, gram-negative bacteria which colonize the gastrointestinal tracts of mammals. Several species colonize the stomach, most notably, *H. pylori*, *H. heilmanii*, *H. felis*, and *H. mustelae*. Although *H. pylori* is the species most commonly associated with human infection, *H. heilmanii* and *H. felis* have also been found to infect humans, but at lower frequencies than *H. pylori*.

Helicobacter infects over 50% of adult populations in developed countries, and nearly 100% in developing countries and some Pacific rim countries, making it one of the most prevalent infections of humans worldwide. Infection with *H. pylori* results in chronic stomach inflammation in all infected subjects, although the clinical gastroduodenal diseases associated with *Helicobacter* generally appear from several years to several decades after the initial infection. *H. pylori* is the causative agent of most peptic ulcers and chronic superficial (type B) gastritis in humans. *H. pylori* infection is also associated with atrophy of the gastric mucosa, gastric adenocarcinoma, and non-Hodgkin's lymphoma of the stomach (see, *e.g.*, Blaser, J. Infect. Dis. 161:626-633, 1990; Scolnick *et al.*, Infect. Agents Dis. 1:294-309, 1993; Goodwin *et al.*, "*Helicobacter pylori*," *Biology and Clinical Practice*, CRC Press, Boca Raton, FL, 465 pp, 1993; Northfield *et al.*, "*Helicobacter pylori*," *Infection*, Kluwer Acad. Pub., Dordrecht, 178 pp, 1994).

If untreated, *H. pylori* infection and the associated gastritis persist lifelong, despite systemic

and local immune responses to the bacterium in the infected host (Crabtree *et al.*, "Host responses," *in Helicobacter pylori Infection*, Northfield *et al.* (Eds.), Kluwer Acad. Pub., Dordrecht, pp. 40-52, 1991; Kist "Immunology of *Helicobacter pylori*," *in Helicobacter pylori in peptic ulceration and gastritis*, Marshall *et al.* (Eds.), Blackwell Sci. Pub., Oxford, pp. 92-110, 1991; Fox *et al.*, Infect. Immun. 61:2309-2315, 1993). Conventional treatment of peptic ulcer disease associated with *H. pylori* infection involves the use of one or more antibiotics combined with a proton pump inhibitor or an H_2-receptor antagonist. Such treatment regimens are unsuccessful in 10% to 70% of patients. Moreover, successful eradication of *H. pylori* infection with antibiotics does not prevent subsequent reinfection. The most effective conventional treatment is a triple therapy with bismuth, metronidazole, and either amoxicillin or tetracycline. The triple therapy treatment is complicated by a complex and prolonged dosing regimen, a high incidence of side-effects, poor compliance, and emergence of resistant bacterial strains (Hentschel *et al.*, N. Engl. J. Med. 328:308-312, 1993).

Summary of the Invention

We have identified a novel *Helicobacter* polypeptide, designated *Helicobacter* ClpB, which may be used, *e.g.*, in methods and compositions for preventing and/or treating *Helicobacter* infection.

Accordingly, the invention features a method for preventing or treating *Helicobacter* infection in a mammal involving administering to the mammal *Helicobacter* ClpB, or an immunogenic fragment or derivative thereof. In this method, administration may be to a mucosal (*e.g.*, intranasal, oral, ocular, gastric, rectal, vaginal, intestinal, or urinary tract) surface of the mammal, or

parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, or intramuscular). In addition to *Helicobacter* ClpB (or an immunogenic fragment or derivative thereof), an adjuvant, *e.g.*, a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), or a fragment or derivative thereof having adjuvant activity, may be administered to the mammal. Mammals that may be treated using the method of the invention include, but are not limited to, mammals such as humans, cows, horses, pigs, dogs, cats, sheep, and goats.

The invention also features a method of preventing or treating *Helicobacter* infection in a mammal involving administering to a mucosal (*e.g.*, oral) surface of the mammal an antibody (*e.g.*, a monoclonal antibody) which recognizes *Helicobacter* ClpB.

Substantially pure *Helicobacter* ClpB polypeptide, for example, a polypeptide containing amino acid sequences substantially identical to the amino acid sequences shown in Fig. 2 (SEQ ID NO:2), is also included in the invention. (The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences in Fig. 2 contain errors, for example, a stop codon is present at amino acid 752. Thus, the invention features a polypeptide having an amino acid sequence which is substantially identical to the ClpB amino acid sequence encoded by the insert in the plasmid contained in the bacteria deposited with the NCIMB as XLOLR HP CP6, see below). Purified DNA which encodes *Helicobacter* ClpB polypeptide is also included in the invention. The purified DNA of the invention may contain a nucleotide sequence which is substantially identical to that of the insert in the plasmid contained in the bacteria deposited with the NCIMB as XLOLR HP CP6, see below. The invention also features a nucleotide sequence which is substantially identical to the nucleotide sequence shown in Fig. 2 (SEQ

ID NO:1). The DNA may be contained in a vector, for example, a plasmid vector, and/or a cell.

The invention also includes a vaccine composition containing, in addition to *Helicobacter* ClpB (or immunogenic fragment or derivative thereof), a pharmaceutically acceptable diluent or carrier (*e.g.*, water, a saline solution (*e.g.*, phosphate-buffered saline), or a bicarbonate solution (*e.g.*, 0.24 M $NaHCO_3$)). An adjuvant, *e.g.*, a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), or a fragment or derivative thereof having adjuvant activity, may also be included in the vaccine composition of the invention.

A method of producing a recombinant *Helicobacter* ClpB polypeptide is also included in the invention. In this method, a cell transformed with DNA encoding *Helicobacter* ClpB polypeptide, and positioned for expression in said cell, is cultured under conditions for expressing the DNA. Recombinant *Helicobacter* ClpB polypeptide is then isolated from the cell. *Helicobacter* ClpB polypeptide made by this method is also included in the invention.

A substantially pure antibody (*e.g.*, a monoclonal antibody, *e.g.*, an IgA or IgG antibody) that specifically binds *Helicobacter* ClpB polypeptide, or a fragment or derivative thereof, is also featured in the invention. This antibody may be used in a method for detecting *Helicobacter* in a sample involving contacting the sample with the antibody and detecting immune complexes formed between the antibody and the sample as an indication of the presence of *Helicobacter* in the sample.

By a "substantially identical" polypeptide sequence is meant an amino acid sequence which differs only by conservative amino acid substitutions, for example, substitution of one amino acid for another of

the same class (*e.g.*, valine for glycine, arginine for lysine, etc.) or by one or more non-conservative substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the function (*e.g.*, the specific antigenicity) of the polypeptide.

Preferably, such a sequence is at least 85%, more preferably 90%, and most preferably 95% identical at the amino acid level to the sequences of Fig. 2 (SEQ ID NO:2), or the ClpB sequence encoded by the insert of XLOLR HP CP6 (see below). For polypeptides, the length of comparison sequences will generally be at least 15 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably at least 35 amino acids.

Homology is typically measured using sequence analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation).

By "substantially pure" is meant a preparation which is at least 60% by weight (dry weight) the compound of interest, *e.g.*, the ClpB polypeptide (or fragment or derivative thereof) or ClpB-specific antibody.

Preferably the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, *e.g.*, column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "purified DNA" is meant DNA that is not immediately contiguous with both of the sequences (*e.g.*, the coding sequences) with which it is immediately contiguous (one on the 5′ end and one on the 3′ end) in the naturally-occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By a "substantially identical" nucleic acid is meant a nucleic acid sequence which encodes a polypeptide differing only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (*e.g.*, valine for glycine, arginine for lysine, etc.) or by one or more non-conservative substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the function of the polypeptide. Preferably, the encoded sequence is at least 45%, more preferably 60%, and most preferably 85% identical at the amino acid level to the amino acid sequences of Fig. 2 (SEQ ID NO:2) (or the amino acid sequence encoded by the insert in XLOLR HP CP6, see below). If nucleic acid sequences are compared a "substantially identical" nucleic acid sequence is one

which is at least 85%, more preferably 90%, and most preferably 95% identical to the sequence of Fig. 2 (SEQ ID NO:1) (or the ClpB amino acid sequence encoded by the insert in XLOLR HP CP6, see below). The length of nucleic acid sequence comparison will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides. Again, homology is typically measured using sequence analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

By "purified antibody" is meant antibody which is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, antibody.

By "specifically binds" is meant an antibody which recognizes and binds a ClpB polypeptide but which does not substantially recognize and bind other molecules in a sample (*e.g.*, a biological sample) which naturally includes ClpB polypeptide. An antibody which "specifically binds" ClpB is sufficient to detect a ClpB protein product in such a biological sample using one or more of the standard immunological techniques available to those in the art (for example, Western blotting or immunoprecipitation).

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (*i.e.*, facilitates the production of *Helicobacter* ClpB protein).

Other features and advantages of the invention will be apparent from the following detailed description

of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first described.

Drawings

Fig. 1 is a schematic representation of the nucleotide sequence of the insert of plasmid pCP6 (SEQ ID NO:1), which insert encodes *Helicobacter* clpB (SEQ ID NO:2). The putative promoter is indicated by the arrow at position 5. The ribosome binding site (RBS; position 116), start codon (ATG; position 126), and stop codon (TGA; position 2695), are indicated. The numbering starts at the beginning of the pCP6 insert. The bold arrow indicates the 5′ to 3′ orientation of the gene.

Fig. 2 is a schematic representation of the nucleotide sequence of (SEQ IDD NO:1), and the amino acid (SEQ ID NO:3) encoded by, the insert of plasmid pCP6, which insert encodes *Helicobacter* ClpB. The start (ATG) and stop (TAG) codons are indicated by the boxes.

Methods and Compositions for Preventing and/or Treating *Helicobacter* Infection

We have isolated a *Helicobacter* gene that encodes a polypeptide (designated *Helicobacter* ClpB) of approximately 87 kD which, based on amino acid sequence comparisons, is a member of the Clp protein family. This family consists of three sub-groups: ClpA, ClpB, and ClpC, which, although differing in size and sequence organization, share extensive regions of homology. Several of the Clp proteins identified thus far are heat shock proteins (hsps), which are thought to function as regulatory subunits of ATP-dependent proteases, and as molecular chaperones.

Helicobacter ClpB can be used in vaccination methods for preventing and/or treating *Helicobacter* (*e.g.*, *H.pylori*, *H. felis*, or *H. heilmanii*) infection in a mammal (*e.g.*, a human). In these methods, *Helicobacter* ClpB, or an immunogenic fragment or derivative thereof, is administered to a mucosal (*e.g.*, intranasal, oral, ocular, gastric, rectal, vaginal, intestinal, and urinary tract) surface of a mammal, or is administered parenterally (*e.g.*, by intravenous, subcutaneous, intraperitoneal, or intramuscular routes). Any of a number of adjuvants that are known to one skilled in the art may be co-administered with the *Helicobacter* ClpB polypeptide. For example, a cholera toxin (CT) or the heat-labile enterotoxin of *Escherichia coli* (LT), or a fragment or derivative thereof having adjuvant activity, may be used in mucosal administration. An adjuvant such as RIBI (ImmunoChem, Hamilton, MT) or aluminum hydroxide may be used in parenteral administration.

Helicobacter ClpB polypeptides which may be used in the vaccination methods of the invention may be prepared using any of several standard methods. For example, standard recombinant DNA methods may be employed (see, *e.g.*, Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., 1994). In these methods, a suitable host cell is transformed with an appropriate expression vector containing all or part of a *Helicobacter* ClpB-encoding nucleic acid (*e.g.*, DNA or RNA) fragment (see Fig. 2 for the nucleotide (SEQ ID NO:2) and amino acid sequences of *Helicobacter* ClpB; also bacteria strain XLOLR HP CP6, which was deposited with the NCIMB (see below) contains a DNA sequence encoding *Helicobacter* ClpB). Nucleic acid fragments encoding *Helicobacter* ClpB may be obtained from plasmid pCP6, which was deposited in a bacterial strain (*E. coli* XLOLR; Stratagene, Ltd., Cambridge, UK) with the National

Collections of Industrial & Marine Bacteria (NCIMB) in Aberdeen, Scotland, on July 4, 1995, and designated with NCIMB accession number 40748. XLOLR HP CP6 contains a vector, designated pCP6, which is a pBK-CMV plasmid vector (Stratagene, Ltd., Cambridge, UK) containing the *H. pylori* ClpB gene inserted into the *BamH1* site of the polylinker. pCP6 was obtained by excision from a bacteriophage vector Lambda Zap II Express (Stratagene, Inc., Cambridge, UK) containing the *H. pylori* ClpB gene. Standard methods of molecular biology may also be used to obtain the *Helicobacter* ClpB gene (see below, and Ausubel *et al.*, *supra*).

Any of a variety of expression systems may be used to produce recombinant *Helicobacter* ClpB polypeptides. For example, ClpB polypeptides may be produced in a prokaryotic host (*e.g.*, *E. coli*) or in a eukaryotic host (*e.g.*, yeast cells (*e.g.*, *Saccharomyces cerevisiae*), mammalian cells (*e.g.*, COS1, NIH3T3, or JEG3 cells), or arthropod cells (*e.g.*, *Spodoptera frugiperda* (SF9) cells)). Such cells are available from a number of different sources known to those skilled in the art, *e.g.*, the NCIMB or the American Type Culture Collection (ATCC), Rockville, MD (also see, *e.g.*, Ausubel *et al.*, *supra*). The transfection/transformation method used, and the choice of expression vector, will depend on the host system selected, as is described by, *e.g.*, Ausubel *et al.*, *supra*. Expression vectors (*e.g.*, plasmid or viral vectors) can be chosen from, *e.g.*, those described in *Cloning Vectors: A Laboratory Manual* (Pouwels *et al.*, 1985, Supp. 1987; also see, *e.g.*, Ausubel *et al.*, *supra*).

Helicobacter ClpB polypeptides, particularly short fragments, may also be produced by chemical synthesis, *e.g.*, by the method described in *Solid Phase Peptide Synthesis*, 1984, 2nd edn., Stewart *et al.*, Eds., Pierce Chemical Co., Rockford, IL, and by standard *in vitro*

translation methods. In addition, *Helicobacter* ClpB may be purified from *Helicobacter* cultures, using standard methods.

In addition to native, full length, *Helicobacter* ClpB, polypeptide fragments of ClpB, or ClpB polypeptides (or polypeptide fragments of ClpB) containing mutations, may be used in the invention, provided that the antigenicity of the polypeptide is retained. Fragments of *Helicobacter* ClpB polypeptides are made by standard methods, including, *e.g.*, recombinant, chemical synthetic, or proteolytic methods (see, *e.g.*, Ausubel *et al.*, *supra*). Generally, ClpB polypeptide fragments for use in the methods of the invention should be at least 12 amino acids in length, in order to maintain antigenicity. Genes encoding fragments of *Helicobacter* ClpB, and/or ClpB polypeptides containing mutations, are made using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*).

Fragments and derivatives of *Helicobacter* ClpB which are included in the invention may be screened for antigenicity and/or therapeutic efficacy using standard methods in the art, *e.g.*, by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity, using, *e.g.*, the *H. felis*-mouse model system (see, *e.g.*, Czinn *et al.*, Vaccine 11(6):637-642, 1993; Lee *et al.*, European Journal of Gastroenterology and Hepatology 7:303-309, 1995).

Fusion proteins containing *Helicobacter* ClpB (or a fragment or derivative thereof) fused to, *e.g.*, an adjuvant (*e.g.*, cholera toxin (CT) or the *Escherichia coli* heat-labile enterotoxin (LT), or a fragment or derivative thereof having adjuvant activity), are also included in the invention, and can be prepared using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*). In addition, the vaccines of the invention may be covalently

coupled or chemically cross-linked to adjuvants (see, *e.g.*, Cryz *et al.*, Vaccine 13:67-71, 1994; Liang *et al.*, J. Immunology 141:1495-1501, 1988; and Czerkinsky *et al.*, Infection and Immunity 57:1072-1077, 1989).

The amount of vaccine administered depends on, *e.g.*, the particular vaccine antigen, whether an adjuvant is co-administered with the antigen, the type of adjuvant co-administered, the mode and frequency of administration, and the desired effect (*e.g.*, protection and/or treatment), as can be determined by one skilled in the art. In general, the vaccine antigens of the invention are administered in amounts ranging between, *e.g.*, 1 µg and 100 mg. If adjuvants are administered with the vaccines, amounts ranging between, *e.g.*, 1 ng and 1 mg may be used. Administration is repeated as necessary, as can be determined by one skilled in the art. For example, a priming dose can be followed by 3 booster doses at weekly intervals.

Vaccine compositions of the invention contain ClpB polypeptide, or immunogenic fragments or derivatives thereof, in a pharmaceutically acceptable carrier or diluent (*e.g.*, water, a saline solution (*e.g.*, phosphate-buffered saline), or a bicarbonate solution (*e.g.*, 0.24 M $NaHCO_3$)). The carriers and diluents used in the invention are selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field, and in the USP/NF. Adjuvants may also be included in these compositions.

Antibodies against *Helicobacter* ClpB may be used in passive immunization methods for protecting and/or treating mammals (*e.g.*, humans) from *Helicobacter* (*e.g.*,

H. pylori, *H. felis*, or *H. heilmanii*) infection. Monoclonal antibodies against *Helicobacter* ClpB are produced using standard immunological methods (see, *e.g.*, Coligan et *al.*, Eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, New York, 1994). Antigens for use in these methods may be obtained, *e.g.*, by expression of the *Helicobacter* ClpB gene in, *e.g.*, *E. coli*, using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*). Antibodies of any isotype, *e.g.*, IgA and IgG, may be used in the invention. In addition to monoclonal antibodies, purified polyclonal antibodies, single chain antibodies, chimeric antibodies (*e.g.*, human/murine chimeric antibodies), humanized antibodies (*e.g.*, humanized murine monoclonal antibodies), and Fab fragments which recognize *Helicobacter* ClpB may be used in the invention. Antibodies which recognize *Helicobacter* ClpB may be identified using standard immunological assays, *e.g.*, Western blot analysis and ELISA (see, *e.g.*, Coligan *et al.*, *supra*). Antibodies may be screened for therapeutic efficacy using, *e.g.*, the *H. felis*-mouse model (see, *e.g.*, Czinn *et al.*, *supra*; Lee *et al.*, *supra*).

In the passive immunization methods of the invention, antibodies (*e.g.*, monoclonal antibodies) which recognize *Helicobacter* ClpB are administered to a mucosal (*e.g.*, oral or intragastric) surface of a mammal. The amount of antibody to be used in this method is readily determined by one skilled in the art.

The ClpB polypeptides, nucleic acids, and antibodies of the invention may also be used for detecting the presence of anti-*Helicobacter* antibodies, *Helicobacter* nucleic acids, or *Helicobacter* polypeptides, respectively, in biological samples, using standard methods (*e.g.*, Western blot analysis, ELISA, and nucleic acid hybridization methods; see, *e.g.*, Ausubel *et al.*,

supra; Coligan *et al., supra*). Thus, nucleic acid fragments, *e.g.*, RNA or DNA fragments of at least 10, preferably at least 12, more preferably at least 15, and most preferably at least 18 nucleotides, which hybridize to nucleic acid which encodes *Helicobacter* ClpB, may be used in diagnostic methods, and are included in the invention.

<u>Isolation of the *Helicobacter* ClpB Gene</u>

The following methods were used to isolate the gene encoding *Helicobacter* ClpB.

<u>Methods</u>:

Preparation of Chromosomal DNA and Construction of a Genomic Library

Genomic DNA was prepared from *H. pylori* NCTC 11637 (ATCC accession number 43504) using the method described by Leying *et al.* (Mol. Microbiol. 6:2863-74, 1992). An expression library containing this *H. pylori* genomic DNA was constructed in the bacteriophage vector Lambda Zap II Express (Stratagene, Inc., Cambridge, UK) by ligating size-selected (2-10 kb) *Sau3A* partially-digested fragments of the genomic DNA into *BamH1*-digested lambda arms. The ligated DNAs were then packaged into phage heads *in vitro*. Analysis of a random selection of clones showed that the average insert size was 4.5 kb.

Preparation of Antiserum

Rabbit antiserum was prepared against *H. pylori* by standard methods using live, whole cells of *H. pylori* Roberts strain as antigens (Luke *et al.*, FEMS Microbiol. Lett. 71:225-230, 1990; Roberts strain was obtained from Dr. C. Penn, School of Biological Sciences, University of Birmingham, Birmingham, UK).

Screening of the Gene Library

The bacteriophage library was plated on *E. coli* XL1-Blue MRF (Stratagene, Ltd., Cambridge, UK) to give approximately 1.4 x 10^3 plaques per 90 mm plate (4.0 x 10^3 plaques in total). The plaques were lifted onto nitrocellulose and screened with a 1:200 dilution of anti-*H. pylori* whole cell antiserum, using standard methods (see, *e.g.*, Maniatis *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). Immunodetection was performed using a mouse anti-rabbit IgG alkaline phosphatase-conjugated antibody (Sigma Chemical Co., St. Louis, MO).

***In Vivo* Excision of Recombinant Plasmid pBK-CMV from Lambda Zap II Express**

In vivo excision of recombinant pBK-CMV from Lambda Zap II Express was carried out according to the methods provided by the manufacturer (Stratagene, Ltd., Cambridge, UK). *E. coli* XLOLR (Stratagene, Ltd., Cambridge, UK) was used for plating excised phagemids.

SDS-PAGE and Immunoblotting

E. coli XLOLR clones were harvested from LB broth cultures, washed in PBS, and resuspended in cracking buffer (49 mM Tris, pH 6.7, containing 2.5% SDS, 1.3% 2-mercaptoethanol, 5% glycerol, and 0.002% bromophenol blue). Samples were boiled for 5 minutes, and 1-2 μg of protein was applied to an SDS polyacrylamide gel. SDS-PAGE and immunoblotting were carried out using standard methods (see, *e.g.*, Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Creation of Nested Deletions

Nested deletions were created using the Erase-A-Base kit (Promega Corporation, Madison, WI), according to the instructions provided by the manufacturer.

Hybridization

A 1.1 kb internal *HindIII* fragment was excised from low melting point agarose and radiolabelled with ^{32}P-dCTP (Amersham, UK) using the Megaprime kit (Amersham, UK), according to the instructions provided by the manufacturer. This fragment was used to probe *HaeIII*-digested genomic DNA from both *cagA*+ and *cagA*- strains of *H. pylori* (*H. pylori* strains 84, 114, 20, 74, and 113 from St. Bartholomew's Hospital, London; and strains 945 and 781 from St. James's Hospital, Dublin).

<u>Results</u>:

Screening of the Library and Identification of CP6

A total of 4 x 10^3 bacteriophages were screened, and 54 clones which reacted with the antiserum were identified. One clone, designated CP6, which expressed an immunoreactive polypeptide of approximately 87 kD, was selected for further study. Plasmid DNA (designated pCP6) was rescued from this clone, as is described above, and was found to contain an insert of 3.2 kb.

Localization of the Gene and DNA Sequence Analysis

A series of nested deletions were created in CP6 with insert sizes ranging from 3-0.3 kb. Immunoblot analysis revealed that clones with insert sizes of less than 3 kb no longer expressed the 87 kD protein, indicating that most of the insert DNA contained coding sequence.

Analysis of the Nucleotide Sequence

A total of 2,755 basepairs of the insert were sequenced. An open reading of 2,571 basepairs was identified, coding for a protein of 857 amino acids. The predicted molecular weight of this protein is 94 kD, which is close to the estimate of 87 kD, which was based on electrophoretic mobility. A schematic representation of the nucleotide sequence of the gene is shown in Fig. 1 (SEQ ID NO:1). Numbering starts at the beginning of the

insert. The structural gene has an ATG initiation codon at position 126 and a stop codon at position 2695. A potential ribosome binding site is present six bases upstream from the start codon. The regions with the closest homology to the *E. coli* σ70 promoter sequences were identified (TTGAGA at position 5 and TATTTT at position 26). The G+C content of the gene is 41%, which is slightly higher than the G+C content (34 to 37%) reported for the entire *H. pylori* genome (Maniatis *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Analysis of the Predicted Amino Acid Sequence

Comparison of the predicted amino acid sequence with known protein sequences on the Swiss protein database (using the BLAST program; see, *e.g.*, Ausubel *et al.*, *supra*) showed a high degree of homology with the Clp family of proteins which are thought to function as molecular chaperones and as ATPase subunits of ATP-dependent proteases. Several of these proteins are listed in Table 1. The Clp proteins share two large, highly conserved sequence blocks, each centered around a nucleotide-binding domain. Alignment of the *H. pylori* predicted amino acid sequence with these proteins showed that the *H. pylori* sequence has a high level of identity with the Clp proteins in the two conserved sequence blocks (Table 1) and also contains two potential nucleotide binding regions.

Hybridization analysis

DNA fragments from 7 different strains of *H. pylori* (3 *cagA*+ and 4 *cagA*-) hybridized with a CP6 probe, and marked restriction fragment length polymorphism was observed.

Table 1. Clp proteins related to the *H. pylori* ClpB amino acid sequence

Organism	Protein name	Length (No. of amino acids)	% identity with *H. pylori* predicted amino acid sequence (in conserved blocks)
Escherichia coli	ClpB	857	65
Bacteroides nodosus	ClpB	860	65
Trypanosoma brucei	Clp	868	61
Pisum sativum (pea)	ClpA	922	63
Lycopersicon esculentum (tomato)	CD4B	923	63
Lycopersicon esculentum (tomato)	CD4A	926	63
Saccharomyces cerevisiae	HSP7	811	62
Saccharomyces cerevisiae	MSP104	908	50

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: OraVax, Inc.

(ii) TITLE OF INVENTION: HELICOBACTER CLPB

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/499,222
(B) FILING DATE: 07-JUL-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: CLARK, PAUL T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 06132/017WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/542-5070
(B) TELEFAX: 617/542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2755 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATATTGAGA GTTGTCTAAA AAGCGTATTT TGGAGAGTYT GTCATTAATG TGAGCGTTTT 60

AAAAACCTTT GAGGGTTAAA ATAGTGTAAA ATAGTAAAGA TTTTAAAACT CAAAAAGGAT 120

TGATAATCAA TTTATTTGAA AAAATGACTG ACCAATTGCA TGAGGCTTTA GACAGCGCGC 180

TCGCTTTAGC TTTACACCAT AAAAACGCTG AAGTAACGCC CTTGCACATG CTTTTTGCCA 240

TGCTCAATAA CTCCCAAGGC ATCCTCATTC AAGCCTTACA AAAAATGCCT GTGGATATTG 300

AAGCTTTAAA ACTTAGCGTT CAAAGCGAGC TGAATAAGTT TGCTAAAGTT TCACAAATCA 360
ATAAGCAAAA TATCCAATTA AACCAAGCTC TAATCCAAAG TTTAGAAAAC GCTCAAGGCT 420
TGATGGCTAA AACGGGCGAT TCTTTCATCG CTACCGATGT GTATCTTTTG GCGAACATGA 480
GCCTTTTTGA AAGCGTTTTA AAGCCTTATT TAGACACTAA GGAATTGCAA AAAACTTTAG 540
AATCTTTAAG AAAAGGCGCG ACTATTCAAG GTAAAAGCGA TGATTCTAAT TTGGAAAGTT 600
TAGAAAAATT TGGCATTGAT TTGACGCAAA AAGCCTTAGA AAATAAGCTG GATCCCGTGA 660
TCGGAAGAGA TGAAGAAATC ATTCGCATGA TGCAAATTTT GATCAGAAAA ACAAAAAATA 720
ACCCTATTTT ACTGGGCGAG CCTGGAGTGG TGAAAACGGC GGTTGTGGAA GGGTTAGCCC 780
AACGCATTGT GAATAAGGAA GTGCCTAAAA CGCTTTTAAA CAAACGAGTC ATCGCTTTAG 840
ATTTAAGCTT GTTGGTGGCT GGAGCGAAAT ACAGAGGCGA GTTTGAAGAG CGCCTGAAAA 900
AGGTGATTGA AGAAGTTAAA AAAAGCGCGA ATGTGATTTT ATTCATTGAT GAAATCCACA 960
CGATCGTAGG GGCTGGGGCT AGTGAGGGGG GCATGGATGC GGCTAATATT TTAAAACCCG 1020
CGCTCGCTAG GGGGGAATTG CACACGATTG GAGCGACCAC CTTGAAAGAA TACCCCAAGT 1080
ATTTTGAAAA AGACATGGCG CTACAAAGGC GTTTCCAACC CATTTTACTC AATGAGCCTA 1140
GCATCAATGA AGCTTTACAG ATTTTAAGGG GGTTAAAAGA AACTTTAGAA ACGCACCATA 1200
ATATCACCAT CAATGACTCC GCGCTCATAG CGAGCGCTAA ACTCTCTAGC CGTTATATCA 1260
CCGATAGGTT TTTACCCGAT AAAGCGATTG ATTTGATTGA TGAGGGGGCG GCTCAATTAA 1320
AAATCCAAAT GGAATCAGAG CCGGCCAAAC TCTCTAGCGT GAAGCGCTCC ATTCCAAGAC 1380
TGGAAATGGA AAAACAAGCC CTTGAAATGG AAAAAAAGGA AAGCAACCAT AAACGCATGC 1440
AAGAAATCCT TAAAGAATTG AGCGATTTGA AAGAAGAAAA AATCCAATTA GAAGCGCAAT 1500
TTGAAAACGA AAAAGAAGCG TTCAAAGAAA TTTCACGCTT GAAAATGGAA ATGGAAAGCT 1560
TGAAAAAAGA GGCTGAGAGG TTTAAGCGCA ATGGGGATTA CCAGCAAGCG GGTGAAATTG 1620
AATACTCTAA AATCCCTGAA AATHAAAAGA AAGAAGAAGA ATTGCAACGT AAATGGGAAG 1680
CGATGCAACA AAACGGGGCG TTGTTGCAAA ACGCTTTAAC CGAAAACAAC ATCGCTGAGA 1740
TCGTGAGCCA ATGGACGCAT ATCCCGGTCC AAAAAATGCT CCAAAGCGAA AAAAATAGGG 1800
TTTTAAACAT TGAAAGCGAA TTGCAAAAAA GAGTGGTGGG GCAAGAAAAA GCGATCAAAG 1860
CGATCGCTAA AGCGATTAAA AGGAATAAGG CCGGACTTAG CGATAGCAAT AAACCCATAG 1920
GGAGTTTCCT CTTTTTAGGG CCAACAGGCG TGGGTAAAAC CGAGAGCGCT AAAGCCTTGG 1980
CGCAATTCTT GTTTGATAGC GATAAAAATC TTATAAGAAT TGACATGAGC GAATATTTGG 2040
AAAAGCATGC CATAAGCCGT CTTATTGGGC CCGCTCCTGG GTATGTGGGC TATGAAGAAG 2100
GCGGGCAGTT GACCGAAGCG GTGCGCAGAA AGCCTTATAG CGTGGTGCTG TTAGATGAAG 2160
TGGAAAAAGC CCATCCAGAT GTGTTTAACC TCTTGTTGCA GGTTTTAGAT GAAGGGCATT 2220

TAACCGATAG TAAGGGCGTG AGGGTGGATT TCAAAAACAC GATTTTGATT TTAACTAGCA 2280

ATGTAGCTAG CGGCGCGCTT TTGGAAGAAA ATTTGAGCGA AGCCGCCAAA CAAAAAGCGA 2340

TTAAAGAGAG CTTGAGCAAT TCTTCAAGCC GGAATTTTTA AACCGCTTAG ATGAAATCAT 2400

CTCCTTTAAC GCCCTAGATA GTCATGCTGT CATTAATATC GTGGGGATAC TCTTTGAAAA 2460

CATTCAAAAA AAAGCGCTTG AAAGGGGCAT TAATATAACT TTAGACGAAG AGGCAAAAGA 2520

ATTGATCGCT GAAGCGGGAT TTGACAGATT TTATGGCGCT AGACCCCTAA AGCGTGCACT 2580

CTATGAAATG GTAGAAGACA AGCTCGSTGA ACTCATTTTA GAGGATAAAA TTAAAGAGAA 2640

TGGSAGCGKG GGATYWGYGG CAGAAMATCA CGAGATTGTG CCTAAGATTA AGTGAAGTCT 2700

GGCTATCCTC AAAANTAAGA AATGGTCATT TTGRGGAAAA GGATTGCAAT GATGT 2755

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 751 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Leu Phe Glu Lys Met Thr Asp Gln Leu His Glu Ala Leu Asp
1 5 10 15

Ser Ala Leu Ala Leu Ala Leu His His Lys Asn Ala Glu Val Thr Pro
20 25 30

Leu His Met Leu Phe Ala Met Leu Asn Asn Ser Gln Gly Ile Leu Ile
35 40 45

Gln Ala Leu Gln Lys Met Pro Val Asp Ile Glu Ala Leu Lys Leu Ser
50 55 60

Val Gln Ser Glu Leu Asn Lys Phe Ala Lys Val Ser Gln Ile Asn Lys
65 70 75 80

Gln Asn Ile Gln Leu Asn Gln Ala Leu Ile Gln Ser Leu Glu Asn Ala
85 90 95

Gln Gly Leu Met Ala Lys Thr Gly Asp Ser Phe Ile Ala Thr Asp Val
100 105 110

Tyr Leu Leu Ala Asn Met Ser Leu Phe Glu Ser Val Leu Lys Pro Tyr
115 120 125

Leu Asp Thr Lys Glu Leu Gln Lys Thr Leu Glu Ser Leu Arg Lys Gly
130 135 140

Ala Thr Ile Gln Gly Lys Ser Asp Asp Ser Asn Leu Glu Ser Leu Glu
145 150 155 160

Lys Phe Gly Ile Asp Leu Thr Gln Lys Ala Leu Glu Asn Lys Leu Asp
165 170 175

Pro Val Ile Gly Arg Asp Glu Glu Ile Ile Arg Met Met Gln Ile Leu
180 185 190

Ile Arg Lys Thr Lys Asn Asn Pro Ile Leu Leu Gly Glu Pro Gly Val
195 200 205

Val Lys Thr Ala Val Val Glu Gly Leu Ala Gln Arg Ile Val Asn Lys
210 215 220

Glu Val Pro Lys Thr Leu Leu Asn Lys Arg Val Ile Ala Leu Asp Leu
225 230 235 240

Ser Leu Leu Val Ala Gly Ala Lys Tyr Arg Gly Glu Phe Glu Glu Arg
245 250 255

Leu Lys Lys Val Ile Glu Glu Val Lys Lys Ser Ala Asn Val Ile Leu
260 265 270

Phe Ile Asp Glu Ile His Thr Ile Val Gly Ala Gly Ala Ser Glu Gly
275 280 285

Gly Met Asp Ala Ala Asn Ile Leu Lys Pro Ala Leu Ala Arg Gly Glu
290 295 300

Leu His Thr Ile Gly Ala Thr Thr Leu Lys Glu Tyr Pro Lys Tyr Phe
305 310 315 320

Glu Lys Asp Met Ala Leu Gln Arg Arg Phe Gln Pro Ile Leu Leu Asn
325 330 335

Glu Pro Ser Ile Asn Glu Ala Leu Gln Ile Leu Arg Gly Leu Lys Glu
340 345 350

Thr Leu Glu Thr His His Asn Ile Thr Ile Asn Asp Ser Ala Leu Ile
355 360 365

Ala Ser Ala Lys Leu Ser Ser Arg Tyr Ile Thr Asp Arg Phe Leu Pro
370 375 380

Asp Lys Ala Ile Asp Leu Ile Asp Glu Gly Ala Ala Gln Leu Lys Ile
385 390 395 400

Gln Met Glu Ser Glu Pro Ala Lys Leu Ser Ser Val Lys Arg Ser Ile
405 410 415

Pro Arg Leu Glu Met Glu Lys Gln Ala Leu Glu Met Glu Lys Lys Glu
420 425 430

Ser Asn His Lys Arg Met Gln Glu Ile Leu Lys Glu Leu Ser Asp Leu
435 440 445

Lys Glu Glu Lys Ile Gln Leu Glu Ala Gln Phe Glu Asn Glu Lys Glu
450 455 460

Ala Phe Lys Glu Ile Ser Arg Leu Lys Met Glu Met Glu Ser Leu Lys
465 470 475 480

Lys Glu Ala Glu Arg Phe Lys Arg Asn Gly Asp Tyr Gln Gln Ala Gly
485 490 495

Glu Ile Glu Tyr Ser Lys Ile Pro Glu Asn Xaa Lys Lys Glu Glu Glu
500 505 510

Leu Gln Arg Lys Trp Glu Ala Met Gln Gln Asn Gly Ala Leu Leu Gln
515 520 525

Asn Ala Leu Thr Glu Asn Asn Ile Ala Glu Ile Val Ser Gln Trp Thr
530 535 540

His Ile Pro Val Gln Lys Met Leu Gln Ser Glu Lys Asn Arg Val Leu
545 550 555 560

Asn Ile Glu Ser Glu Leu Gln Lys Arg Val Val Gly Gln Glu Lys Ala
565 570 575

Ile Lys Ala Ile Ala Lys Ala Ile Lys Arg Asn Lys Ala Gly Leu Ser
580 585 590

Asp Ser Asn Lys Pro Ile Gly Ser Phe Leu Phe Leu Gly Pro Thr Gly
595 600 605

Val Gly Lys Thr Glu Ser Ala Lys Ala Leu Ala Gln Phe Leu Phe Asp
610 615 620

Ser Asp Lys Asn Leu Ile Arg Ile Asp Met Ser Glu Tyr Leu Glu Lys
625 630 635 640

His Ala Ile Ser Arg Leu Ile Gly Pro Ala Pro Gly Tyr Val Gly Tyr
645 650 655

Glu Glu Gly Gly Gln Leu Thr Glu Ala Val Arg Arg Lys Pro Tyr Ser
660 665 670

Val Val Leu Leu Asp Glu Val Glu Lys Ala His Pro Asp Val Phe Asn
675 680 685

Leu Leu Leu Gln Val Leu Asp Glu Gly His Leu Thr Asp Ser Lys Gly
690 695 700

Val Arg Val Asp Phe Lys Asn Thr Ile Leu Ile Leu Thr Ser Asn Val
705 710 715 720

Ala Ser Gly Ala Leu Leu Glu Glu Asn Leu Ser Glu Ala Ala Lys Gln
725 730 735

Lys Ala Ile Lys Glu Ser Leu Ser Asn Ser Ser Ser Arg Asn Phe
740 745 750

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Glu Leu Glu Gln Phe Phe Lys Pro Glu Phe Leu Asn Arg Leu Asp
1 5 10 15

Glu Ile Ile Ser Phe Asn Ala Leu Asp Ser His Ala Val Ile Asn Ile
20 25 30

Val Gly Ile Leu Phe Glu Asn Ile Gln Lys Lys Ala Leu Glu Arg Gly
35 40 45

Ile Asn Ile Thr Leu Asp Glu Glu Ala Lys Glu Leu Ile Ala Glu Ala
50 55 60

Gly Phe Asp Arg Phe Tyr Gly Ala Arg Pro Leu Lys Arg Ala Leu Tyr
65 70 75 80

Glu Met Val Glu Asp Lys Leu Xaa Glu Leu Ile Leu Glu Asp Lys Ile
85 90 95

Lys Glu Asn Xaa Ser Xaa Gly Xaa Xaa Ala Glu Xaa His Glu Ile Val
100 105 110

Pro Lys Ile Lys
115

What is claimed is:

1. A vaccine for preventing *Helicobacter* infection in a mammal, said vaccine comprising *Helicobacter* ClpB, or an immunogenic fragment or derivative thereof, formulated for administration.

2. The vaccine of claim 1, wherein said mucosal surface is intranasal.

3. The vaccine of claim 1, wherein said mucosal surface is oral.

4. The vaccine of claim 1, wherein said *Helicobacter* ClpB, or said immunogenic fragment or derivative thereof, comprises an adjuvant.

5. The vaccine of claim 4, wherein said adjuvant is selected from the group consisting of a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), and fragments and derivatives thereof having adjuvant activity.

6. A vaccine for treating *Helicobacter* infection in a mammal, said vaccine comprising *Helicobacter* ClpB, or an immunogenic fragment or derivative thereof, formulated for therapeutic administration to a mucosal surface of said mammal.

7. The vaccine of claim 6, wherein said mucosal surface is intranasal.

8. The vaccine of claim 6, wherein said mucosal surface is oral.

9. The vaccine of claim 5, wherein said *Helicobacter* ClpB, or said immunogenic fragment or derivative thereof, comprises an adjuvant.

10. The vaccine of claim 9, wherein said adjuvant is selected from the group consisting of a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), and fragments and derivatives thereof having adjuvant activity.

11. A composition for preventing *Helicobacter* infection in a mammal, said composition comprising an antibody which recognizes *Helicobacter* ClpB, formulated for administration to a mucosal surface of said mammal.

12. The composition of claim 11, wherein said mucosal surface is oral.

13. A composition for treating *Helicobacter* infection in a mammal, said composition comprising an antibody which recognizes *Helicobacter* ClpB, formulated for administration to a mucosal surface of said mammal.

14. The composition of claim 13, wherein said mucosal surface is oral.

15. Substantially pure *Helicobacter* ClpB polypeptide.

16. The polypeptide of claim 15, wherein said polypeptide comprises an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 2.

17. Purified DNA encoding the polypeptide of claim 15.

18. The purified DNA of claim 17, wherein said purified DNA comprises a nucleotide sequence substantially identical to the nucleotide sequence shown in Fig. 2.

19. A vector comprising the purified DNA of claim 17.

20. A cell comprising the purified DNA of claim 17.

21. A vaccine comprising the polypeptide of claim 15 in a pharmaceutically acceptable carrier or diluent.

22. A method of producing a recombinant *Helicobacter* ClpB polypeptide, said method comprising the steps of:

a. providing a cell transformed with DNA encoding said *Helicobacter* ClpB polypeptide, said DNA being positioned for expression in said cell;

b. culturing said transformed cell under conditions for expressing said DNA; and

c. isolating said recombinant *Helicobacter* ClpB polypeptide.

23. *Helicobacter* ClpB polypeptide produced by the method of claim 22.

24. A substantially pure antibody that specifically binds *Helicobacter* ClpB polypeptide.

25. The substantially pure antibody of claim 24, wherein said antibody is a monoclonal antibody.

26. A method of detecting *Helicobacter* in a sample, said method comprising the steps of:

a. contacting said sample with the antibody of claim 24; and

b. detecting immune complexes formed between said antibody and said sample as an indication of the presence of *Helicobacter* in said sample.

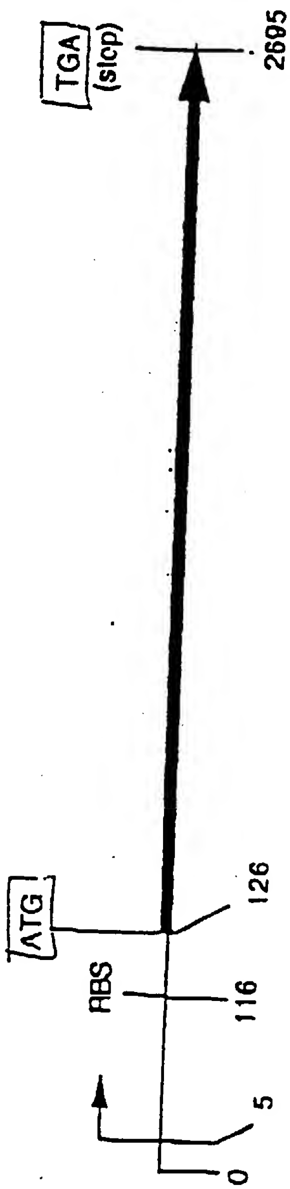

Fig. 1

```
1    AATATTGAGAGTTGTCTAAAAAGCGTATTTGGAGAGTTTGTCATTAATGTGAGCGTT
59   TTAAAAACCTTTGAGCCTTAAAATAGTGTAAAATAGTAAAGATTTTAAAACTCAAAAAGGATTCATA
     Met Asn Leu Phe Glu Lys Met Thr Asp Gln Leu His Glu Ala Leu Asp Ser    17
126  ATG AAT TTA TTT CAA AAA ATG ACT GAC CAA TTG CAT GAG GCT TTA GAC AGC
     Ala Leu Ala Leu Ala Leu His His Lys Asn Ala Glu Val Thr Pro Leu His    34
177  GCG CTC GCT TTA GCT TTA CAC CAT AAA AAC GCT GAA GTA ACG CCC TTG CAC
     Met Leu Phe Ala Met Leu Asn Asn Ser Gln Gly Ile Leu Ile Glu Ala Leu    51
228  ATG CTT TTT GCC ATG CTC AAT AAC TCC CAA GGC ATC CTC ATT GAA GCC TTA
     Gln Lys Met Pro Val Asp Ile Glu Ala Leu Lys Leu Ser Val Glu Ser Glu    68
279  CAA AAA ATG CCT CTG GAT ATT GAA GCT TTA AAA CTT AGC GTT CAA AGC CAG
     Leu Asn Lys Phe Ala Lys Val Ser Glu Ile Asn Lys Gln Asn Ile Gln Leu    85
330  CTG AAT AAG TTT GCT AAA GTT TCA CAA ATC AAT AAG CAA AAT ATC CAA TTA
     Asn Gln Ala Leu Ile Gln Ser Leu Glu Asn Ala Gln Gly Leu Met Ala Lys    102
381  AAC CAA GCT CTA ATC CAA AGT TTA GAA AAC GCT CAA GGC TTG ATG GCT AAA
     Thr Gly Asp Ser Phe Ile Ala Thr Asp Val Tyr Leu Leu Ala Asn Met Ser    119
432  ACG GGC GAT TCT TTC ATC GCT ACC GAT GTG TAT CTT TTG GCG AAC ATG AGC
     Leu Phe Glu Ser Val Leu Lys Pro Tyr Leu Asp Thr Lys Glu Leu Glu Lys    136
483  CTT TTT GAA AGC GTT TTA AAG CCT TAT TTA GAC ACT AAG GAA TTG CAA AAA
     Thr Leu Glu Ser Leu Arg Lys Gly Ala Thr Ile Gln Gly Lys Ser Asp Asp    153
534  ACT TTA GAA TCT TTA AGA AAA GGC GCG ACT ATT CAA GGT AAA AGC GAT GAT
     Ser Asn Leu Glu Ser Leu Glu Lys Phe Gly Ile Asp Leu Thr Gln Lys Ala    170
585  TCT AAT TTG GAA AGT TTA GAA AAA TTT GGC ATT GAT TTG ACG CAA AAA GCC
     Leu Glu Asn Lys Leu Asp Pro Val Ile Gly Arg Asp Glu Glu Ile Ile Arg    187
636  TTA GAA AAT AAG CTC GAT CCC GTG ATC GGA AGA GAT GAA GAA ATC ATT CGC
     Met Met Gln Ile Leu Ile Arg Lys Thr Lys Asn Asn Pro Ile Leu Leu Gly    204
687  ATG ATG CAA ATT TTG ATC AGA AAA ACA AAA AAT AAC CCT ATT TTA CTG GGC
     Glu Pro Gly Val Val Lys Thr Ala Val Val Glu Gly Leu Ala Gln Arg Ile    221
738  CAG CCT GGA GTG GTG AAA ACG GCG GTT GTG GAA GGG TTA GCC CAA CGC ATT
     Val Asn Lys Glu Val Pro Lys Thr Leu Leu Asn Lys Arg Val Ile Ala Leu    238
789  GTC AAT AAG GAA GTG CCT AAA ACG CTT TTA AAC AAA CGA GTC ATC GCT TTA
     Asp Leu Ser Leu Leu Val Ala Gly Ala Lys Tyr Arg Gly Glu Phe Glu Glu    255
840  GAT TTA AGC TTG TTG GTG GCT GGA GCG AAA TAC AGA GGC GAG TTT GAA GAG
     Arg Leu Lys Lys Val Ile Glu Glu Val Lys Lys Ser Ala Asn Val Ile Leu    272
891  CGC CTG AAA AAG GTG ATT GAA GAA GTT AAA AAA AGC GCC AAT GTG ATT TTA
     Phe Ile His Glu Ile His Thr Ile Val Gly Ala Gly Ala Ser Glu Gly Gly    289
942  TTC ATT CAT GAA ATC CAC ACG ATC GTA GGG GCT GGG GCT AGT GAG GGG GGC
     Met Asp Ala Ala Asn Ile Leu Lys Pro Ala Leu Ala Arg Gly Glu Leu His    306
993  ATG GAT GCG GCT AAT ATT TTA AAA CCC GCG CTC GCT AGG GGC GAA TTG CAC
     Thr Ile Gly Ala Thr Thr Leu Lys Glu Tyr Pro Lys Tyr Phe Glu Lys Asp    323
1044 ACG ATT GGA GCG ACC ACC TTG AAA GAA TAC CCC AAG TAT TTT GAA AAA GAC
     Met Ala Leu Gln Arg Arg Phe Gln Pro Ile Leu Leu Asn Glu Pro Ser Ile    340
1095 ATG GCC CTA CAA AGG CGT TTC CAA CCC ATT TTA CTC AAT GAG CCT AGC ATC
     Asn Glu Ala Leu Gln Ile Leu Arg Gly Leu Lys Glu Thr Leu Glu Thr His    357
1146 AAT GAA GCT TTA CAG ATT TTA AGG GGG TTA AAA GAA ACT TTA GAA ACG CAC
     His Asn Ile Thr Ile Asn Asp Ser Ala Leu Ile Ala Ser Ala Lys Leu Ser    374
1197 CAT AAT ATC ACC ATC AAT GAC TCC GCG CTC ATA GCG AGC GCT AAA CTC TCT
     Ser Arg Tyr Ile Thr Asp Arg Phe Leu Pro Asp Lys Ala Ile Asp Leu Ile    391
1248 AGC CGT TAT ATC ACC GAT AGG TTT TTA CCC GAT AAA GCG ATT GAT TTG ATT
     Asp Glu Gly Ala Ala Gln Leu Lys Ile Gln Met Glu Ser Glu Pro Ala Lys    408
1299 GAT GAG GGC GCG GCT CAA TTA AAA ATC CAA ATG GAA TCA GAG CCC GCC AAA
     Leu Ser Ser Val Lys Arg Ser Ile Pro Arg Leu Glu Met Glu Lys Gln Ala    425
1350 CTC TCT AGC GTG AAG CGC TCC ATT CCA AGA CTG GAA ATG GAA AAA CAA GCC
     Leu Glu Met Glu Lys Lys Glu Ser Asn His Lys Arg Met Gln Glu Ile Leu    442
1401 CTT GAA ATG GAA AAA AAG GAA AGC AAC CAT AAA CGC ATG CAA GAA ATC CTT
     Lys Glu Leu Ser Asp Leu Lys Glu Glu Lys Ile Gln Leu Glu Ala Gln Phe    459
1452 AAA GAA TTG AGC GAT TTG AAA GAA GAA AAA ATC CAA TTA GAA GCG CAA TTT
     Glu Asn Glu Lys Glu Ala Phe Lys Glu Ile Ser Arg Leu Lys Met Glu Met    476
1503 GAA AAC GAA AAA GAA GCG TTC AAA GAA ATT TCA CGC TTG AAA ATG GAA ATG
     Glu Ser Leu Lys Lys Glu Ala Glu Arg Phe Lys Arg Asn Gly Asp Tyr Gln    493
1554 GAA AGC TTG AAA AAA GAG GCT GAG AGG TTT AAG CGC AAT GGG GAT TAC CAG
     Gln Ala Gly Glu Ile Glu Tyr Ser Lys Ile Pro Glu Asn Xaa Lys Lys Glu    510
1605 CAA GCG GGT GAA ATT GAA TAC TCT AAA ATC CCT GAA AAT HAA AAG AAA GAA
     Glu Glu Leu Gln Arg Lys Trp Glu Ala Met Gln Gln Asn Gly Ala Leu Leu    527
1656 GAA GAA TTG CAA CGT AAA TGG GAA GCG ATG CAA CAA AAC GGG GCG TTG TTG
     Gln Asn Ala Leu Thr Glu Asn Asn Ile Ala Glu Ile Val Ser Gln Trp Thr    544
1707 CAA AAC GCT TTA ACC GAA AAC AAC ATC GCT GAG ATC GTC AGC CAA TGG ACG
     His Ile Pro Val Gln Lys Met Leu Gln Ser Glu Lys Asn Arg Val Leu Asn    561
1758 CAT ATC CCG GTC CAA AAA ATG CTC CAA AGC GAA AAA AAT CGC GTT TTA AAC
     Ile Glu Ser Glu Leu Gln Lys Arg Val Val Gly Gln Glu Lys Ala Ile Lys    570
```

Fig. 2

1 AATATTGAGAGTTGTCTAAAAACCGTATTTTGGAGAGTYTGTCATTAATGTGAGCGTTTTAAAAAC
67 CTTTGAGGGTTAAAATAGTGTAAAATAGTAAAGATTTTAAAACTCAAAAAGGATTGATAATGAATTT
134 ATTTGAAAAAATGACTGACCAATTCCATGAGGCTTTAGACAGCGCGCTCCCTTTAGCTTTACACCAT
201 AAAAACGCTGAACTAACGCCCTTGCACATGCTTTTTGCCATGCTCAATAACTCCCAAGGCATCCTCA
268 TTCAAGCCTTACAAAAAATGCCTGTCGATATTGAAGCTTTAAAACTTAGCGTTCAAAGCGAGCTGAA
335 TAAGTTTGCTAAACTTTCACAAATCAATAAGCAAAATATCCAATTAAACCAAGCTCTAATCCAAAGT
402 TTAGAAAACGCTCAAGGCTTGATCCCTAAAACGGGCGATTCTTTCATCCCTACCGATGTGTATCTTT
469 TGGCGAACATGACCCTTTTTGAAAGCGTTTTAAAGCCTTATTTAGACACTAAGGAATTGCAAAAAAC
536 TTTAGAATCTTTAAGAAAAGCCCCGACTATTCAAGGTAAAAGCGATGATTCTAATTTGGAAAGTTTA
603 GAAAAATTTGCCATTGATTTGACGCAAAAAGCCTTAGAAAATAAGCTGGATCCCGTGATCCCAAGAG
670 ATGAAGAAATCATTCGCATGATGCAAATTTTGATCAGAAAAACAAAAAATAACCCTATTTTACTGG
737 CGAGCCTGGAGTCCTGAAAACGGCGGTTGTGGAAGCGGTTACCCCAACGCATTGTGAATAAGGAACTC
804 CCTAAAACGCTTTTAAACAAACGAGTCATCGCTTTAGATTTAAGCTTGTTGGTGCCTGGAGCGAAAT
871 ACAGAGGCGAGTTTGAACAGCGCCTGAAAAAGGTGATTGAAGAAGTTAAAAAAAGCGCGAATGTGAT
938 TTTATTCATTGATGAAATCCACACGATCGTAGGGGCTGGGGCTAGTGAGGGGGGCATGGATGCGGCT
1005 AATATTTTAAAACCCCCGCTCGCTAGGGGGGAATTGCACACGATTGGAGCGACCACCTTGAAACAAT
1072 ACCCCAAGTATTTTGAAAAAGACATCGCGCTACAAAGGCGTTTCCAACCCATTTTACTCAATGAGCC
1139 TAGCATCAATGAAGCTTTACAGATTTTAAGGGGGTTAAAAGAAACTTTAGAAACGCACCATAATATC
1206 ACCATCAATGACTCCGCGCTCATAGCGACCGCTAAACTCTCTAGCCGTTATATCACCGATAGGTTTT
1273 TACCCGATAAAGCGATTCATTTGATTGATGAGGGGCCGGCTCAATTAAAAATCCAAATGGAATCAGA
1340 GCCCCCCAAACTCTCTAGCGTGAAGCGCTCCATTCCAAGACTGGAAATGGAAAAACAAGCCCTTGAA
1407 ATGGAAAAAAAGGAAACCAACCATAAACGCATGCAAGAAATCCTTAAAGAATTGAGCGATTTGAAAC
1474 AAGAAAAAAATCCAATTAGAAGCGCAATTTCAAAACGAAAAAGAAGCGTTCAAAGAAATTTCACGCTT
1541 GAAAATGGAAATGGAAACCTTGAAAAAAGAGGCTGAGAGGTTTAAGCGCAATGGGATTACCAGCAA
1608 GCGGGTCAAATTGAATACTCTAAAATCCCTGAAAATHAAAAGAAAGAAGAAGAATTGCAACGTAAAT
1675 GGGAAGCGATGCAACAAAACGGGGCGTTGTTGCAAAACGCTTTAACCGAAAACAACATCGCTGAGAT
1742 CGTGAGCCAATGGACGCATATCCCCGGTCCAAAAAAATGCTCCAAAGCGAAAAAAATAGCCTTTTAAAC
1809 ATTGAAAGCGAATTGCAAAAAAGAGTGGTGGCGCAAGAAAAAGCCATCAAAGCGATCGCTAAAGCCA
1876 TTAAAAGCAATAAGGCCGGACTTAGCGATAGCAATAAACCCATAGGGAGTTTCCTCTTTTTAGGGCC
1943 AACAGGCGTGGGTAAAACCCAGAGCGCTAAAGCCTTGGCGCAATTCTTGTTTGATAGCGATAAAAAT
2010 CTTATAACAATTGACATGAGCGAATATTTGCAAAAGCATGCCATAAGCCGTCTTATTGGCCCCGCTC
2077 CTGGGTATGTGGGCTATCAAGAAGGCGGGCAGTTGACCGAAGCCGTGCGCAGAAAGCCTTATAGCGT
2144 GGTGCTGTTAGATGAAGTGGAAAAAGCCCATCCAGATGTGTTTAACCTCTTGTTGCACCTTTTAGAT
2211 GAAGGGCATTTAACCGATACTAAGGGCGTGAGGGTGGATTTCAAAAACACGATTTTGATTTTAACTA
2278 GCAATGTACCTAGCGGCGCGCTTTTGGAAGAAAATTTGAGCGAAGCTGCCAAACAAAAACCGATTAA
Arg Glu Leu Glu Gln Phe Phe Lys Pro Glu Phe Leu Asn Arg Leu Asp Glu
2345 AGA GAG CTT GAG CAA TTC TTC AAG CCG CAA TTT TTA AAC CGC TTA GAT GAA 17
Ile Ile Ser Phe Asn Ala Leu Asp Ser His Ala Val Ile Asn Ile Val Gly
2396 ATC ATC TCC TTT AAC GCC CTA GAT AGT CAT GCT GTC ATT AAT ATC GTG CGG 34
Ile Leu Phe Glu Asn Ile Gln Lys Lys Ala Leu Glu Arg Gly Ile Asn Ile
2447 ATA CTC TTT GAA AAC ATT CAA AAA AAA CCG CTT GAA AGG GGC ATT AAT ATA 51
Thr Leu Asp Glu Glu Ala Lys Glu Leu Ile Ala Glu Ala Gly Phe Asp Arg
2498 ACT TTA GAC GAA GAG GCA AAA CAA TTC ATC GCT GAA GCG GGA TTT CAC AGA 68
Phe Tyr Gly Ala Arg Pro Leu Lys Arg Ala Leu Tyr Glu Met Val Glu Asp
2549 TTT TAT CGC GCT AGA CCC CTA AAG CCT GCA CTC TAT GAA ATG GTA CAA GAC 85
Lys Leu Xxx Glu Leu Ile Leu Glu Asp Lys Ile Lys Glu Asn Xxx Ser Xxx
2600 AAG CTC CST GAA CTC ATT TTA GAG GAT AAA ATT AAA GAG AAT GGS ACC GKG 102
Gly Xxx Xxx Ala Glu Xxx His Glu Ile Val Pro Lys Ile Lys Stop
2651 GGA TYW GYC GCA GAA MAT CAC GAG ATT GTG CCT AAG ATT AAG TGA AGTCTGG 116
2703 CTATCCTCAAAAMTAAGAAATGCTCATTTTGRGGAAAAGGATTGCAATGATGT

Fig. 2 cont.

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---- ATT GAA AGC GAA TTG CAA AAA AGA GTG GTG GGG CAA GAA AAA GCG ATC AAA
     Ala Ile Ala Lys Ala Ile Lys Arg Asn Lys Ala Gly Leu Ser Asp Ser Asn     595
1860 GCG ATC GCT AAA CCG ATT AAA AGG AAT AAG GCC GGA CTT AGC GAT AGC AAT
     Lys Pro Ile Gly Ser Phe Leu Phe Leu Gly Pro Thr Gly Val Gly Lys Thr     612
1911 AAA CCC ATA GGG ACT TTC CTC TTT TTA GGG CCA ACA GGC CTG GGT AAA ACC
     Glu Ser Ala Lys Ala Leu Ala Gln Phe Leu Phe Asp Ser Asp Lys Asn Leu     629
1962 CAG AGC GCT AAA GCC TTG GCG CAA TTC TTG TTT GAT AGC GAT AAA AAT CTT
     Ile Arg Ile Asp Met Ser Glu Tyr Leu Glu Lys His Ala Ile Ser Arg Leu     646
2013 ATA AGA ATT GAC ATG AGC GAA TAT TTG GAA AAG CAT GCC ATA AGC CGT CTT
     Ile Gly Pro Ala Pro Gly Tyr Val Gly Tyr Glu Glu Gly Gly Gln Leu Thr     663
2064 ATT GGG CCC GCT CCT GGG TAT GTG GGC TAT GAA GAA GGC GGC CAG TTG ACC
     Glu Ala Val Arg Arg Lys Pro Tyr Ser Val Val Leu Leu Asp Glu Val Glu     680
2115 GAA GCG GTG CGC AGA AAG CCT TAT AGC GTG GTG CTG TTA GAT GAA GTG GAA
     Lys Ala His Pro Asp Val Phe Asn Leu Leu Leu Gln Val Leu Asp Glu Gly     697
2166 AAA GCC CAT CCA GAT GTG TTT AAC CTC TTG TTG CAG GTT TTA GAT GAA GGG
     His Leu Thr Asp Ser Lys Gly Val Arg Val Asp Phe Lys Asn Thr Ile Leu     714
2217 CAT TTA ACC GAT AGT AAG GGC GTG AGG GTC GAT TTC AAA AAC ACG ATT TTG
     Ile Leu Thr Ser Asn Val Ala Ser Gly Ala Leu Leu Glu Glu Asn Leu Ser     731
2268 ATT TTA ACT AGC AAT GTA GCT AGC GGC GCG CTT TTG GAA GAA AAT TTG AGC
     Glu Ala Ala Lys Gln Lys Ala Ile Lys Glu Ser Leu Ser Asn Ser Ser Ser     748
2319 GAA GCC GCC AAA CAA AAA GCG ATT AAA GAG AGC TTG AGC AAT TCT TCA AGC
     Arg Asn Phe Stop                                                        751
2370 CGC AAT TTT TAA ACCGCTTAGATGAAATCATCTCCTTTAACGCCCTAGATAGTCATGCTGTCA
2433 TTAATATCGTGCCGATACTCTTTGAAAACATTCAAAAAAAGCGCTTGAAAGGGGCATTAATATAAC
2500 TTTACACGAAGAGGCAAAAGAATTGATCGCTGAAGCGGGATTTGACAGATTTTATGCCGCTAGACCC
2567 CTAAAGCGTGCACTCTATGAAATGGTAGAAGACAAGCTCGSTGAACTCATTTTAGAGGATAAAATTA
2634 AAGAGAATGGSAGCGKGGGATYWCYGGCAGAAMATCACGAGATTGTGCCTAAGATTAAGTCAAGTCT
2701 GGCTATCCTCAAAANTAAGAAATGGTCATTTTCRGGAAAAGGATTGCAATGATGT
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Fig. 2 cont.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11116

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/554, 33/569
US CL :424/184.1; 435/7.32
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1; 435/7.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, MPSEARCH, CAS ONLINE
search terms: pylori?, chaperone, proteosome, ATP-dependent, vaccin?, heat shock, hsp, clpB,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FERRERO et al. The GroES homolog of *Helicobacter pylori* confers protective immunity against mucosal infection in mice. Proc. Natl. Acad. Sci. USA. 03 July 1995, Vol. 92, No. 14, pages 6499-6503, especially figure 1, page 6500, see whole article.	1-10, 17-23
Y	WO 93/18150 A1 (BIOCINE SCLAVO S.P.A.) 16 September 1993 (16.09.93), page 1, abstract, see whole document.	1-10, 17-22
A	McGHEE et al. The mucosal immune system: from fundamental concepts to vaccine development. Vaccine. 1992, Vol. 10, issue 2, pages 75-88, see whole article.	1-10, 21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 AUGUST 1996	29 AUG 1996

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	CZINN et al. Oral immunization against *Helicobacter pylori*. Infection and Immunity. July 1991, Vol. 39, No. 7, pages 2359-2363, especially pages 2359-2960.	1-10 -------- 26
A	RAPPUOLI et al. Development of a vaccine against *Helicobacter pylori*: a short overview. European Journal of Gastroenterology and Hepatology. 1993, Vol. 5, Supplement 2, pages 576-578, see whole article.	1-10
X --- Y	ENGSTRAND et al. An increased number of γ/δ T-cells and gastric epithelial cell expression of the groEL stress-protein homologue in *Helicobacter pylori*-associated chronic gastritis of the antrum. American Journal of Gastroenterology. August 1991, Vol. 86, No. 8, pages 976-980, especially page 979, figure 3.	15-16, 24-26 -------- 1-10, 22-23
X --- Y	YOKOTA et al. Heat Shock protein produced by *Helicobacter pylori*. Microbiolgoy and Immunology. Vol. 38, No. 5, pages 403-405, especially pages 403 and 405.	15-16, 24-26 -------- 1-10
Y	BUKANOV et al. Ordered cosmid library and high-resolution physical-genetic map of *Helicobacter pylori* strain NCTC11638. Molecular Microbiology. February 1994, Vol. 11, No. 3, pages 509-523; especially pages 515 and 516.	17-23
X --- A	FIGURA, N. Progress in defining the inflammatory cascade. European Journal of Gastroenterology and Hepatology. 1995, Vol. 7, No. 4, pages 296-302, especially pages 297-299.	15-16 -------- 1-26
Y	COPPEL et al. 'Antibody screening of expression libraries.' In: Methods in Molecular Biology. Edited by J.E. Hyde. Totowa, NJ: Humana Press Inc, 1993, Vol. 21, pages 277-296, especially pages 284 and 291-295.	15-26
A	LINDQUIST, S. Heat-shock proteins and stress tolerance in microorganisms. Current Opinion in Genetics and Development. 1992, Vol. 2, pages 748-755, especially table 1.	1-26
A	SAUERBAUM et al. *Helicobacter pylori hspA-hspB* heat-shock gene cluster: nucleotide sequence, expression, putative function and immunogenicity. Molecular Microbiology. 1994, Vol. 14, No. 5, pages 959-74, especially pages 959-960.	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11116

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VIALE et al. Evolutionary relationships among Eubacterial groups as inferred from GroEL (chaperonin) sequence comparisons. International Journal of Systematic Bacteriology. July 1994, Vol. 44, No. 3, pages 527-529 and 531-533, see table 1.	1-26

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